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(54) Title: METHODS FOR PREPARING BAKED GOODS WITH ADDITION OF LIPID-COATED ENZYME

(57) **Abstract:** The present invention provides a novel baked good which has incorporated therein a delivery vehicle which provides protection from thermal denaturation and continuously releases an α-amylase enzyme during and following the baking process. Essentially, dry, food-grade α-amylase particles are mixed with a food grade lipid in a quantity sufficient to envelop the α-amylase particles or aggregates thereby forming a loaded delivery vehicle or enveloped α-amylase. The enveloped α-amylase is subsequently added to a dough comprising flour, sugar, shortening, milk powder, salt, yeast and water near the end of the mix cycle. Once the enveloped α-amylase is incorporated, the dough continues through the normal production process for that particular baked product. The food grade lipid enveloping the α-amylase provides thermal protection to the enzyme. No other production or packaging modifications need be made.

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METHODS FOR PREPARING BAKED GOODS WITH ADDITION OF LIPID-COATED ENZYME

DESCRIPTION

Technical Field

The present invention relates to a method for retarding the staling of baked goods and more particularly to a method for achieving the thermal protection and sustained release of a certain enzyme throughout a baked good during and following the baking process.

Background Art

The phenomenon of bread staling has been studied extensively and a variety of theories have been presented. It is now generally accepted that staling is due to a gradual transition of starch from an amorphous structure to a partially crystalline state. This increase in starch crystallinity, also referred to as retrogradation, is caused by an intermolecular or intra-molecular association, via hydrogen bonding, of the two polysaccharides which comprise starch granules, amylose and amylopectin.

Amylose is made up largely of unbranched chains of D-glucose units (100-1,400 units) which are joined together by α- (1,4)-glucosidic bonds. Retrogradation of amylose is rapid due to the ease of alignment of the linear molecules. Amylopectin is the main constituent of starch and, like amylose, it is also constructed from D-glucose units, but in the case of amylopectin they are assembled in shorter, rather bush like, branched chains, containing only 20-25 units of D-glucose. The links in the chain are α- (1,4)-glucoside bonds, while the branching points involve α- (1,6)-glucosidic bonds. The branched structure of amylopectin interferes with molecular alignment, and consequently amylopectin retrogradation occurs at a much slower rate.

During baking, starch granules swell and absorb moisture, but gelatinization is not complete because of limited water availability. As the granules swell, amylose and to a lesser extent amylopectin diffuse from the granules into the interstitial volume. The solubilized linear molecules retrograde rapidly and form a crystalline network which in combination with the gluten matrix form the characteristic "crumb set" or structure of bread and other baked goods.

Staling of baked goods is generally defined as an increase in crumb firmness and a corresponding loss in product freshness. Flavor, aroma, texture, perceived moisture level, and other product characteristics are also negatively affected as staling proceeds. The staling process begins as soon as baking is complete. Amylopectin remains mostly in the starch granule and retrogrades slowly during product storage. Retrogradation occurs by intermolecular and intra-molecular association of linear segments, and to a lesser extent between amylopectin and amylose at the interface of the starch granules and the interstitial volume. As amylopectin retrogradation proceeds, a three-dimensional crystalline structure is formed slowly, causing an increase in firmness, or staling.

Factors that control the rate of staling include time, temperature, moisture level, and the presence of additives such as emulsifiers (crumb softeners). Rate of staling shows a linear response with time, but can be minimized by maintaining the maximum allowable moisture in the product or by storage at warm (room temperature or higher) or cold (below freezing) temperatures. Refrigeration enhances staling since the rate of retrogradation is optimal at cold temperatures just above freezing.

Staling eventually causes a product to become unacceptable at the retail or consumer level. It is estimated that 3-5% of all baked goods produced in the United States are discarded due to a loss in freshness. The value of discarded baked goods has been estimated to

exceed $1 billion annually in the U.S. alone. It is obvious that prolonging the freshness of baked goods by retarding staling would be a benefit to the producer, retailer, and consumer.

A common practice within the baking industry, to retard staling, is to add chemical emulsifiers to the dough formulation. About 12-15 million pounds of distilled monoglyceride and 20-25 million pounds of mono- and diglycerides are used annually in the baking industry for this purpose. However, while chemical emulsifiers do produce a softer bread, they are only partially effective in reducing bread staling because they appear to function by creating softer bread out of the oven rather than by acting upon the mechanism of starch retrogradation directly—the bread still stales at about the same rate, but it starts from a softer loaf and so reaches unacceptable firmness later than untreated bread. As can be surmised from this description, a limiting factor in surfactant use is the initial softness of the loaf: both bakery production processes (such as slicing), and consumer preferences require a certain level of firmness in bread which sets a limit to surfactant use.

In addition to the usage of chemical emulsifiers, enzymes which modify the starch structure responsible for staling are also used for increasing shelf-life of baked goods. Enzymatic techniques for reducing firming in baked goods have been studied for years, and the beneficial action of enzymes has been recognized. However, commercially available enzymes have been in the past either only marginally effective or they produced offsetting negative effects in product quality that precluded widespread use.

The amylases are a specific type of enzyme which hydrolyze the glycosidic linkages in polyglucans, and for this reason are grouped with hydrolases. The specific amylases of special interest to bakers are α-(1,4)-glucan glucanohydrolase (or α- amylase) and α- (1,4)-glucan maltohydrolase (or beta-amylase) derived from various cereal and

microbial sources. The amylases are widely distributed in nature, occurring in many animal tissues, higher plants, molds, yeast and bacteria. Until recently, the only α- amylases used in baking were cereal enzymes from barley malt, fungal enzymes derived mainly from *Aspergillus oryzae*, and bacterial enzymes derived from *Bacillus subtilis*. Depending on their origin, α- amylases show measurable differences in certain properties, such as pH and temperature optima, thermostability, and resistance to inactivation by acidity. They are simple crystallizable proteins that do not require the presence of coenzymes for their activity. Because of their protein nature, they exhibit a general heat lability. Table I, shown below, demonstrates the thermostability of α- amylases from various sources.

TABLE I

Temperature		Percent of Enzyme Activity		
°C	°F	Fungal	Barley Malt	Bacterial
65	149	100	100	100
70	158	52	100	100
75	167	3	58	100
80	176	1	25	92
85	189	–	1	58
90	194	–	–	22
95	203	–	–	8

The data in Table 1 demonstrates that fungal α- amylase is quite heat labile and is inactivated rapidly at temperatures above 149°F (65°C). A temperature above 167°F (75°C) is required for a comparable inactivation of cereal α- amylase. Bacterial α- amylase is the most stable and shows little loss of activity at temperatures up to 185°F (85°C).

As the temperature of the dough rises during baking, starch is gelatinized over the range of 140° to 167°F (60° to 75°C), rendering it susceptible to amylase attack. A- amylase specifically hydrolyses the α- (1,4)-glycosidic linkages in starch at random points within the amylose and amylopectin molecules. Some α- amylases are capable of hydrolysing linkages within the amorphous regions of the starch matrix during baking. Under the proper conditions, this limited degree of hydrolysis is sufficient to disrupt the starch network and reduce the rate of staling.

Barley malt is often added directly to wheat flour at the mill to standardize α- amylase activity. Standardization enhances production of fermentable sugars from damaged starch, increases yeast growth and gas production, and improves dough handling and proofing. Barley malt also improves finished product properties such as color, grain, texture, and flavor. However, since barley malt retains much of its activity over the temperature range of starch gelatinization, it is important to avoid an excess of cereal amylase to prevent the undesireable result of gummy, sticky crumb. Shelf-life, however is not improved.

Bacterial α- amylase enzyme most often refers to enzymes made from *Bacillus subtilis*, and are able to inhibit staling by hydrolysing glycosidic linkages within the amorphous areas of gelatinized starch. The enzyme is most active at a pH of about 7 and a temperature of about 75 to 80°C.

One enzymatic approach to retarding bread staling is disclosed in U.S. Pat. No. 2,615,810 to Stone and involves the use of a heat-stable bacterial α- amylase enzyme to attack gelatinized starch granules during baking. A refinement to Stone's approach is described in U.S. Pat. No. 4,299,848 to DeStefanis, *et al.* which discloses a process for the inactivation of the proteolytic enzymes present in

commercially available heat stable bacterial α- amylase enzyme preparations obtained from extracts of *Bacillus subtilis, Bacillus stearothermophilus* or other microbial sources. In a further refinement, U.S. Pat. No. 4,654,216 to Carroll, *et al.* discloses the addition of an enzyme mixture of heat stable bacterial α- amylase and a pullulanase to dough in proportions of from 0.25 to 5 SKB (α- amylase units) and 5 to 75 PUN (debranching enzyme units) per 100 grams of flour.

G. Bussiere, *et al.* in "The Utilization of Alpha-Amylase and Glucoamylase in Industrial Baking Technology," *Annales De Technologie Agricole*, volume 23 (2) pages 175 to 189 (1974) discloses studies on the role of heat stable bacterial α- amylases derived from *Bacillus subtilis* in bread making technology. Bussiere, *et al.* teaches that heat stable α- amylases of bacterial origin are effective in retarding staling, but produce a sticky bakery product when used a dosage of 2.5 SKB units or more per 100 grams of flour.

A drawback of the Stone, DeStefanis, *et al.*, Carroll, *et al.*, and Bussiere, *et al.* approaches is the tendency of heat stable bacterial α-amylases to remain active too long during baking and to cause gumminess in the finished product. As a result, these approaches require a degree of control over dosages and enzyme ratios which may be impractical to apply commercially.

Further attempts to improve the action of bacterial amylases have focused on genetic manipulation of the naturally occurring bacteria to create a bacteria which produces alpha-amylase which is less thermostable. Some of these products, such as Novamyl® from Novo Nordisk BioChem (Franklinton, NC) have partially overcome the limitations of naturally occurring bacterial amylase and have achieved some acceptance in the industry, but finished product quality still needs improvement and the reliance on genetic modification makes such products unacceptable for use in "Certified Organic" foods (as defined

by the California Organic Foods Act of 1990) which constitutes a significant developing market for baked goods.

Fungal α- amylase enzymes are effective in partially hydrolysing damaged starch and are often added to flour, in the same manner as barley malt, to develop desirable properties for baking. However, conventional fungal amylases exhibit limited thermostability and are, for the most part, inactivated prior to the onset of starch gelatinization during baking since their optimum temperature range is only 50-55°C. As a result, fungal α- amylases have little effect on amylopectin hydrolysis and do not exhibit significant anti-staling activity.

In an attempt to provide a fungal α- amylase that exhibits anti-staling activity, Cole in his U.S. Patent No. 4,320,131 discloses that the thermal stability of fungal α- amylase is substantially increased by dispersing aqueous solutions of the enzyme in concentrated sugar solutions. This procedure reportedly protects the enzyme from thermal denaturation, allowing it to retain activity during baking. Use of the stabilized enzyme in conjunction with the proper emulsifier in a carefully controlled process reputedly reduces product firmness, although use of the enzyme alone is not effective. However, the processing and ingredient changes required make this approach unsuitable for a number of bakery applications.

There is still a need, therefore, for a method and composition produced therefrom which utilizes α- amylase enzymes in a manner that is suitable in a number of bakery applications and which achieves an acceptable baked good having an extended shelf-life.

Disclosure of Invention

Accordingly, it is an object of this invention to provide a method for retarding the staling of baked goods.

More specifically, it is an object of this invention to provide a delivery vehicle for α- amylase enzymes which protect the enzyme

from thermal denaturation and provides for a sustained release of the enzyme.

It is a further object of this invention to provide a baked good having incorporated within it a delivery vehicle which continuously releases an active α- amylase enzyme during and following the baking process.

It is a further object to accomplish all of the aforementioned objects in baked goods that meet the requirements of the California Organic Foods Act of 1990 or any comparable act.

Additional objects, advantages and novel features of this invention shall be set forth in part in the description that follows, and in part will become apparent to those skilled in the art upon examination of the following specification or may be learned by the practice of the invention. The objects and advantages of the invention may be realized and attained by means of the instrumentalities, combinations, and methods particularly pointed out in the appended claims.

To achieve the foregoing and other objects and in accordance with the purposes of the present invention, as embodied and broadly described therein, the method of this invention results in a baked good comprising flour, water, other dough-forming ingredients, and an effective quantity of a loaded delivery vehicle wherein said loaded delivery vehicle comprises α- amylase particles in surface contact with a food grade lipid.

Best Mode for Carrying out the Invention

The present invention provides a novel baked good which has incorporated therein a delivery vehicle which provides protection from thermal denaturation and continuously releases an α- amylase enzyme during and following the baking process. Essentially, dry, food-grade α- amylase particles are mixed with a food grade lipid in a quantity sufficient to envelop the α- amylase particles or aggregates thereby

forming a loaded delivery vehicle or enveloped α- amylase. The enveloped α- amylase is subsequently added to a dough comprising flour, sugar, shortening, milk powder, salt, yeast and water near the end of the mix cycle. Once the enveloped α- amylase is incorporated, the dough continues through the normal production process for that particular baked product. The food grade lipid enveloping the α-amylase provides thermal protection to the enzyme. No other production or packaging modifications need be made.

The present invention begins with the production of a delivery vehicle containing an α- amylase enzyme. As way of illustration, only fungal α- amylase will be referred to below, however, the person skilled in the art will recognize that a variety of α- amylases are available in the industry, such as, but not limited to bacterial-derived and cereal-derived types. The fungal α- amylase disclosed herein can be obtained from Enzyme Development Corporation, N.Y., N.Y. and is sold under the name ENZECO® Fungal Alpha-Amylase Powder.

First, fungal α- amylase is contacted with a food grade lipid, discussed in further detail below, in sufficient quantity so as to suspend all of the fungal α- amylase. The activity of fungal α- amylase can be expressed in terms of SKB units, and the preferred level of activity is that provided by about 200 - 5,000 SKB units per pound of formula flour. However, both lesser and greater amounts are satisfactory. When very high levels, such as in excess of 200,000 SKB units of enzyme are used, no detrimental effects are noted, but there is a decided leveling off of beneficial enzyme activity, and hence from the cost standpoint, there is a practical upper limit. At less than 200 SKB units, it is difficult to appreciate the improved effects, and therefore, such a level may be considered a practical lower limit, and about 5,000 SKB units may be considered a preferred usage level.

Food grade lipids, as used herein, may be any natural occurring organic compound that is insoluble in water, but soluble in non-polar

organic solvents, such as, hydorcarbon or diethyl ether. The food grade lipids preferably utilized in the present invention include, but are not limited to triglycerides either in the form of fats or oils which are either saturated or unsaturated. Examples of fatty acids and combinations thereof which make up the saturated triglycerides utilized in the present invention include, but are not limited to, the following: butyric (derived from milk fat), palmitic (derived from animal and plant fat), and/or stearic (derived from animal and plant fat). Examples of fatty acids and combinations thereof which make up the unsaturated triglycerides utilized in the present invention include, but are not limited to, the following: palmitoleic (derived from animal and plant fat), oleic (derived from animal and plant fat), linoleic (derived from plant oils), and/or linolenic (derived from linseed oil). Other food grade lipids which are contemplated and within the scope of the present invention include, but are not limited to, monoglycerides and diglycerides derived from the triglycerides discussed above, phospholipids and glycolipids.

The food grade lipid, preferably in the liquid form, is contacted with the α- amylase particles in such a fashion that the lipid material covers at least a portion of the surface of at least a majority of the α-amylase particles. Thus, the particles of α- amylase can all be individually enveloped in the lipid or an aggregate of such particles is enveloped. In the preferred embodiment of the present invention, all or substantially all of the particles of α- amylase are provided with a thin, continuous, enveloping film of lipid. This can be accomplished by first pouring a quantity of lipid into a container and then slurrying the α-amylase so that the lipid thoroughly wets the surfaces of the α-amylase particles. After a short period of stirring, the α- amylase particles, carrying a substantial amount of the lipids on their surfaces, are recovered. The thickness of the coating so applied to the particles of α- amylase can be controlled by selection of the type of lipid used

and by repeating the operation in order to build up a thicker film, when desired.

The storing, handling and incorporation of the loaded delivery vehicle of the present invention is most conveniently accomplished by means of a packaged mix. Preferably, the packaged mix comprises the enveloped α- amylase; however, it is within the teachings of this patent that the packaged mix may further contain additional ingredients as required by the manufacturer or baker. After the enveloped α-amylase has been incorporated into the dough, the baker continues through the normal production process for that product. The advantages of enveloping the α- amylase are two-fold. First, it has been discovered that the food grade lipid protects the enzyme from thermal denaturation during the baking process for those enzymes that are heat labile, such as the fungal α- amylases. See Table II, below.

TABLE II

Temperature		Percent of Enzyme Activity	
°C	°F	Fungal-Unprotected From Table I	Fungal-Protected as disclosed by the present invention
65	149	100	100
70	158	52	100
75	167	3	100
80	176	1	100
85	189	–	100
93	200	–	95
99	210	–	50
114	220	–	25

The illustration of the thermal protection provided by the present invention and summarized in Table II was established by heating a 50:50 mixture of fungal alpha amylase and rapeseed oil to various

temperatures and holding the mixture for five (5) minutes at that elevated temperature and then allowing it to cool back to room temperature. Five (5) ml of the oil:enzyme mix was then placed in 300 ml of 45° C water and stirred for 10 minutes to extract some of the enzyme from its oil coating. The extraction was then allowed to cool back to room temperature. An aliquot of this extraction was then mixed into soft starch gel samples (prepared from corn starch powder), and the change in length of time required to liquify the starch gel to a standard viscosity was used to indicate the level of enzyme activity.

Consequently, while the α- amylase is stabilized, it is also capable of leaching through the protective coating where it hydrolyzes the glucosidic linkages in polyglucons. Surprisingly, the second advantage is that following the baking process, active α- amylase continues to leach out through the protective coating at a rate which counteracts the staling mechanisms.

In general, the amount of lipid applied to the α- amylase particles can vary from a few percent of the total weight of the α-amylase to many times that weight, depending upon the nature of the lipid, the manner in which it is applied to the α- amylase particles, the composition of the dough mixture to be treated, and the severity of the dough-mixing operation involved.

The baker computes the amount of enveloped α- amylase prepared, as discussed above, that will be required to achieve the desired anti-staling effect. The amount of the enveloped α- amylase required is calculated based on the concentration of enzyme enveloped and on the proportion of α- amylase to flour specified by this invention. A wide range of concentrations has been found to be effective, although, as has been discussed, observable improvements in anti-staling do not correspond linearly with the α- amylase concentration, but above certain minimal levels, large increases in α-amylase concentration produce little additional improvement. The α-

amylase concentration actually used in a particular bakery production could be much higher than the minimum necessary in order to provide the baker with some insurance against inadvertent under-measurement errors by the baker. The lower limit of enzyme concentration is determined by the minimum anti-staling effect the baker wishes to achieve, and is not integral to the invention itself.

The enveloped α- amylase is added to the dough near the end of the mix cycle. Depending on the type and volume of dough, and mixer action and speed, anywhere from one to six minutes or more might be required to mix the enveloped α- amylase into the dough, but two to four minutes is average. There are several variables here that determine the precise procedure: First, the quantity of enveloped α-amylase must have a total volume sufficient to allow the enveloped α-amylase to be spread throughout the dough mix. (If the preparation of enveloped α- amylase is highly concentrated, additional oil may need to be added to the pre-mix before the enveloped α- amylase is added to the dough.) Recipes and production processes may require specific modifications; however, good results have been achieved when 25% of the oil specified in a bread dough formula is held out of the dough and is used as a carrier for a concentrated enveloped α- amylase when added near the end of the mix cycle. In bread/baked goods, recipes which have extremely low fat content (such as french breads), it has been found that an enveloped α- amylase mixture of approximately 1% of the dry flour weight is sufficient to properly admix the enveloped α-amylase with the dough, but the range of percentages that may work is extremely wide and is dependent on the formula, finished-product, and production methodology requirements of the individual baker rather than upon any known limitations of the invention. Second, the enveloped α- amylase suspension must be added to the mix with enough time remaining in the mix cycle for complete mixture into the dough, but not so early that excessive mechanical action will strip a

large proportion of the enveloped α- amylase from its protective coating.

A typical and satisfactory recipe according to the present invention is provided for in Table III below:

TABLE III

Ingredient	Amount added to dough batch of 100 lbs. of flour
Canola (Rapeseed oil) (lipid)	1 g
fungal α amylase	25,000 SKB units

The foregoing description is considered as illustrative only of the principles of the invention. Furthermore, since numerous modifications and changes will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and processes shown as described above. Accordingly, all suitable modifications and equivalents may be resorted to falling within the scope of the invention as defined by the claims which follow. The words "comprise," "comprises," "comprising," "include," "including," and "includes," when used in this specification and in the following claims are intended to specify the presence of stated features, integers, components, or steps, but they do not preclude the presence of addition of one or more other features, integers, components, steps, or groups thereof.

Claims

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method for preparing a baked good comprising combining flour or meal, water, other dough-forming ingredients, and an effective quantity of a loaded delivery vehicle wherein said loaded delivery vehicle comprises α- amylase particles in surface contact with a lipid.

2. The method of claim 1, wherein said α- amylase particles as derived from a fungus.

3. The method of claim 1, wherein said α- amylase are particles derived from a cereal.

4. The method of claim 1, wherein said α- amylase particles are derived from a bacteria.

5. The method of claim 2, wherein said fungus is *Aspergillus oryzae*.

6. The method of claim 3, wherein said cereal is barley malt.

7. The method of claim 4, wherein said bacteria is *Bacillus subtilis*.

8. The method of claim 1, wherein substantially all of said α-amylase particles are completely covered by said lipid.

9. The method of claim 1, wherein said lipid is a triglyceride.

10. The method of claim 11, wherein said triglyceride is a fat.

11. The method of claim 11, wherein said triglyceride is an oil.

12. The method of claim 11, wherein said oil is either saturated or unsaturated.

13. The method of claim 10, wherein said fat is either saturated or unsaturated.

14. The method of claim 5, wherein said α- amylase derived from said fungus has at least 5,000 SKB units per 100 pounds of flour.

15. An article of manufacture which comprises, flour or meal, water, and any other ingredients and an active α- amylase enzyme surrounded by a lipid.

16. The article of claim 14, wherein said active α- amylase enzyme is derived from a fungus.

17. The article of claim 15, wherein said lipid is a triglyceride.

18. The article of claim 17, wherein said triglyceride is an oil.

19. A method for thermally protecting an enzyme by enveloping said enzyme in a lipid.

20. The method of claim 20, wherein said enzyme is a fungal α- amylase.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A21D8/04 C11D3/386 C12N9/96 C12N9/28 C12N9/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A21D A23L C11D C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, EPO-Internal, PAJ, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 32336 A (COTTRELL JOHN ;DALGETY PLC (GB); FRAZIER PETER (GB); SAXBY DAVID ()) 30 July 1998 (1998-07-30) page 5, line 22-36 examples 1,6 claims 1-9,12-20 ---	1,2, 8-13, 15-20
X	GB 1 544 499 A (KYOWA HAKKO KOGYO KK) 19 April 1979 (1979-04-19) page 2, line 114 -page 3, line 96 examples 3,12 claims 1,5,13,15,19,20 ---	1,4, 8-11,15, 17-19

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 3 561 975 A (LUEBERING JAMES E ET AL) 9 February 1971 (1971-02-09) column 2, line 40-64 column 4, line 6-48 column 5, line 18-29 claims 1,4,7,8; example I ---	19
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Information on patent family members

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